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14. ABSTRACT Cancer cells have evolved to evade immune-mediated destruction through several documented mechanisms. Our group has developed a technique to enhance immune function in tumor-bearing hosts by targeting a protein on the surface of white blood cells, termed OX40. This type of immune modulation leads to therapeutic benefit in tumor-bearing mice. We have produced a protein that binds to the human OX40 protein and activates human white blood cells. We have a cell line that produces high quantities of this protein and our goal is to test this protein for safety and efficacy in non-human primates so that we can obtain FDA approval for clinical trials in cancer patients. The long-range goal of this proposal is translate these findings to prostate cancer patients.					
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DOD Prostate Award Research Technical Reporting: Progress Report
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PC101977 – Production and Characterization of a Novel OX40 Ligand for Clinical Use.

PI -Andrew Weinberg, PhD

INTRODUCTION: Cancer cells have evolved to evade immune-mediated destruction through several documented mechanisms. Our group has developed a technique to enhance immune function in tumor-bearing hosts through the use of OX40 agonists, which can lead to regression of tumors of various histologies, including prostate cancer. In particular, we have produced a human OX40 agonist, termed OX40L:ILZ:Ig (OX40L:Ig) that has potent biologic function in vitro and is produced in large quantities by tissue culture cells. The ILZ portion of the chimeric protein was initially a trimerization domain obtained from a yeast sequence. In the past few years we have produce a fully human OX40 ligand protein and it was tested for in vivo biologic activity in non-human primates and had potent activity. The ultimate goal of the current research is to produce clinical grade human OX40L:Ig to test in clinical trials for patients suffering from prostate cancer. With that goal in mind we made a GMP compliant cell line that produces large quantities of the protein within the first year of funding. Future work will include testing this protein in monkey primates for toxicology studies, which are typically mandated by the FDA prior to approval for phase I studies to be conducted in cancer patients. Ultimately this protein will be tested in cancer patients in a phase I study.

KEYWORDS: Cancer immunotherapy, OX40, T cell costimulation

BODY: The last year of funding was spent on understanding whether anti-tumor efficacy of the OX40L:Ig fusion protein could be enhanced in combination with other immunotherapies. In previous work funded by the DOD we characterized the optimal sequences that gave us the most potent biologic activity for the human OX40L:Ig fusion protein. This protein was subsequently tested in non-human primate studies and the

potent biologic activity observed allowed for confidence to move forward with cell line production.

In the Fall of 2011, we licensed the OX40L:Ig fusion program to an industry sponsor for testing in cancer patients. The license deal is a collaborative project between the two groups and is especially advantageous in terms of increased support for the program as far as taking this platform through phase I, II, and III clinical trials. The sponsor's protein chemistry group has taken several antibodies and Ig fusion proteins to the clinic and has vast experience communicating with the FDA to gain approval for clinical testing. The formulation that was settled on as the product going forward included the same domains that we had put together for the OX40L and isoleucine zipper portions of the construct, but the human Fc tail was changed from IgG1 to IgG4 based on non-human primate studies. These studies showed that the IgG4 chimeric protein had a longer half-life than the IgG1 construct (increased from 12 hrs to 3 days in non-human primates). In September of 2014 a dose escalation study commenced in humans with stage IV cancer using the IgG4 OX40L protein. Unfortunately, the pharmacokinetics in humans did not recapitulate the non-human primate studies and this protein showed a much lower half-life in humans than was observed in monkeys. Hence it took increased amounts of this drug to enhance T cell proliferation in humans as compared to non-human primates. The drug was dosed once every two weeks and the highest dose, 30 mg/kg, showed the greatest T cell activation (see Figure 1). Currently, the patients in the final cohort are being accrued and evaluated in order to determine the next steps with this immune activating drug.

We have been testing immune enhancing agents in combination with the OX40L:Ig fusion protein in order to increase the potency of this immunotherapy. Once the phase I portion of the OX40L:Ig clinical trial is complete having preclinical data to support combination therapy will allow for a fast transition to more potent combination trials in the future. In particular we have focused on a small molecule inhibitor of the TGF- β receptor, which has been shown to enhance T cell function within microenvironments rich in TGF- β (e.g. tumors). We have found that TGF- β receptor blockade and OX40 agonists can have potent immune stimulating effects leading to immune-mediated tumor destruction, however when the tumors become larger ($>50\text{mm}^2$)

both agents are not nearly as therapeutic. Hence we tested whether the OX40L:Ig fusion protein when combined with TGF- β receptor blockade would lead to immune-mediated destruction of large tumors. Figure 2 shows that OX40L:Ig or SM16 alone had limited therapeutic effects in mice harboring large tumors, however when these two agents were combined 61% of the mice completely rejected large tumors. We are currently trying to understand the mechanism(s) of how the combined therapy works (e.g. CD4 vs CD8 T cell involvement) and are looking for biomarkers of response to this potent combination therapy.

KEY RESEARCH ACCOMPLISHMENTS:

- The OX40L:Ig fusion is currently in a dose escalation study in stage IV cancer patients and at the highest dose administered to patients we have observed immune stimulating effects.
- We have combined the OX40L:Ig fusion protein therapy with TGF- β receptor blockade and found therapeutic synergy with this combination in mice harboring large tumors.

REPORTABLE OUTCOMES:

The experiments planned within this proposal are to develop an OX40L:Ig fusion protein that can be injected into cancer patients and this has been accomplished with our corporate partner. We have previously published data showing that OX40 Abs and TGF- β receptor blockade were efficacious and in this report we confirm those effects with the OX40L:Ig fusion protein. In the phase I clinical trial with the OX40L:Ig fusion protein our group was one of many sites and we were monly able to evaluate 3-4 patients each from different cohorts for immune enhancing effects, hence publishing these finding may be difficult.

CONCLUSION:

In summary, we have shown that the OX40L:Ig fusion protein can dramatically enhance T cell activation in cancer patients, albeit at high doses. Due to the short half-life of this protein in humans it may be that the dosing scheme needs to change to

administering this protein three times a week followed by a 30-day rest period prior to retreatment. Hopefully a concentrated dosing scheme will be performed after the completion of the phase I trial. Combining the OX40L:Ig fusion protein with a TGF- β receptor inhibitor increased the immune-mediated therapeutic potential of this therapy leading to destruction of large tumors. Combining these two immunotherapies would most likely lead to increased clinical benefit for cancer patients and we hope that this combination will be tested clinically in the near future.

REFERENCES: N/A

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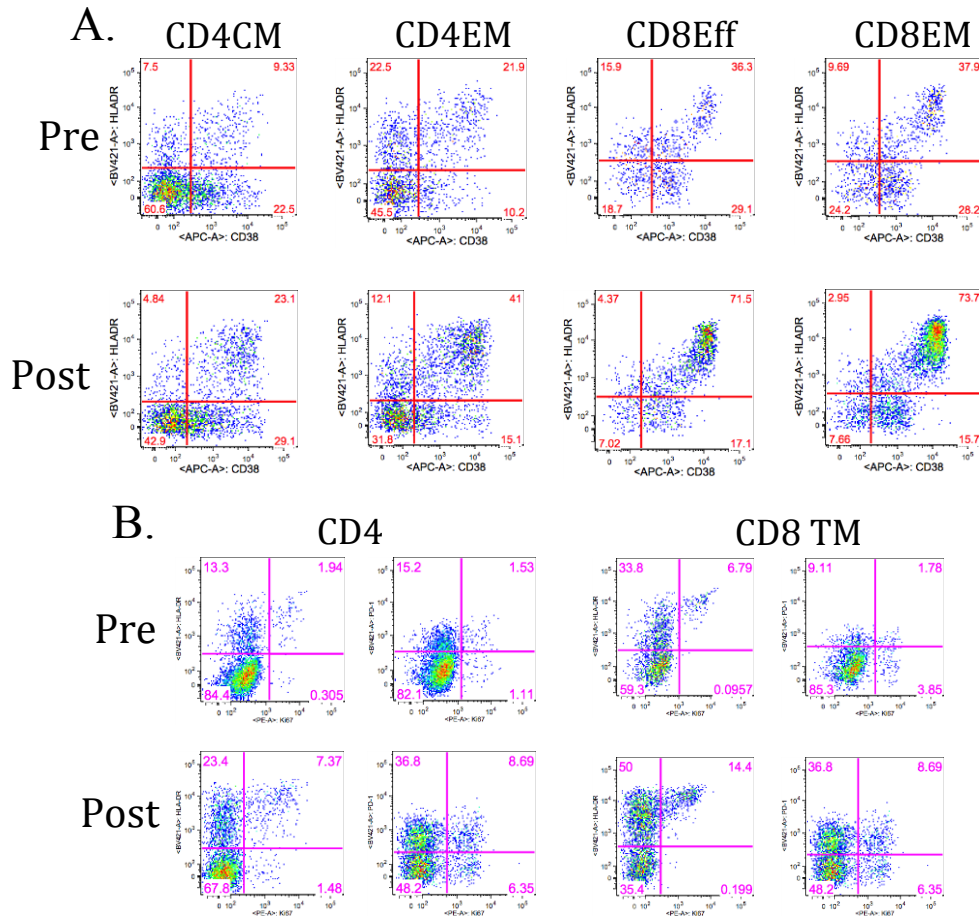


Figure 1: OX40L:Ig therapy increases CD4 and CD8 T cell activation in a cancer patient. This patient was treated with one dose of OX40L:Ig at 30mg/kg and blood was obtained pre and post treatment (7 days after OX40L:Ig). A) CD4 (left) and CD8 (right) T cells were gated on central memory (CM) or effector memory (EM) and assessed for the two activation markers HLA-DR and CD38. B) CD4 (left) and CD8 (right) were gated on total memory (TM) T cells and were assessed for proliferation via Ki-67 expression versus HLA-DR (left panels) and Ki-67 versus PD-1 expression is shown in panels on the right.

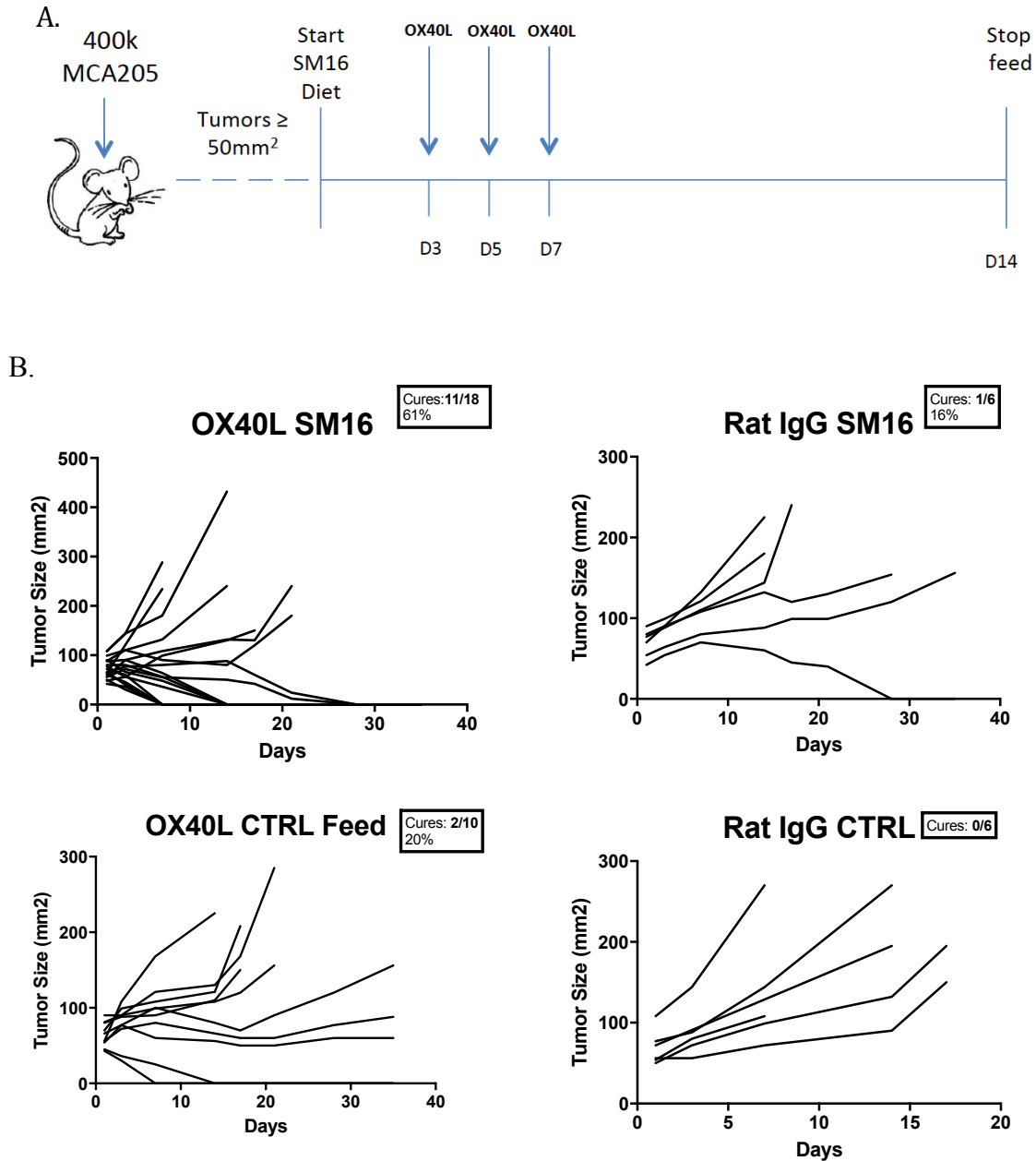


Figure 2: TGF- β receptor inhibition increases therapeutic efficacy of OX40L:Ig treatment. Mice were inoculated with the MCA205 sarcoma (400,000 cells) and treatment commenced when the tumors reached 50mm^2 . The mice were initially treated with 50mg/kg of the TGF- β receptor inhibitor (SM16) in the feed and three days later they received 3 doses of OX40L:Ig (250ug/dose) on days 3, 5 and 7 (A). The mice were kept on SM16 feed for two weeks and all groups were evaluated for tumor growth. B) Tumor growth curves are shown for all the groups and the percentage of mice with complete regression (Cures) is shown in the upper right corner.